Small molecular weight drug-protein interaction measured by MP-SPR.

The interaction of a small molecular weight drug (indomethacin) to Human serum albumin (HSA) was measured with label-free Multi-Parametric Surface Plasmon Resonance (MP-SPR). HSA was attached to a sensor surface with amine coupling chemistry. Affinity (K_D) and kinetic constants (k_a and k_d) of indomethacin interaction were determined with TraceDrawer™ for MP-SPR Navi™. Premium quality kinetic data was ensured using PureKinetics™ feature that separates bulk effect from binding kinetics.

Introduction

Surface Plasmon Resonance (SPR) is an optical phenomenon which is highly sensitive for detecting refractive index changes near the measurement surface, in particular molecular binding or release. Using the SPR phenomenon, Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a real-time and label free *in vitro* tool for molecule-molecule interactions providing information on the affinity and kinetics of the studied system. MP-SPR is suitable for interaction measurements with various systems.

Ligand: nucleotide, peptide, antibody, drug molecule, virus, nanoparticle, microvesicle, or cell

Analyte: protein, nucleotide, peptide, receptor, membrane receptor, drug molecule, antibody, or cell

MP-SPR measures in an advantageously wide angular range, where the complere SPR curve can be monitored in real-time. This enables observing not only SPR peak minimum position but also other parameters such as peak minimum intensity and the total internal reflection (TIR).

PureKinetics™ is a unique tool in MP-SPR measurements, which allows for the correction of the SPR signal due to the discrepancies in buffer composition. This feature is based on the MP-SPR ability to capture complete SPR curves during the interaction measurement. PureKinetics™ is an extremely important feature for the detection of small molecules, when at the same time there are strong bulk changes caused by solubility enhancers such as dimethylsulfoxide (DMSO).

Drug - protein interactions represent a key research area in drug development and protein research. Human serum albumin (HSA) is the most important protein in blood plasma, due to its high concentration and ability to bind various compounds. Main functions of HSA are carrying fatty acids and maintaining blood colloidal osmotic pressure. Additionally, it is an important carrier for many hydrophobic hormones and drugs.

Materials and methods

Carboxymethyl dextran coated sensor slide (BioNavis SPR102- CMD-3D) was used to immobilize HSA with amine coupling chemistry (Figures 1 and 2). The immobilization was performed *in situ* at 21 °C using 5 mM MES (2-(N-morpholino) ethanesulfonic acid) pH=5 as running buffer. Surface was activated with 0.2 M EDC/ 0.05 M NHS before protein injection and deactivated with 1 M ethanolamine pH 8 after protein attachment. HSA sample 0.2 mg/ml was injected only in one of the channels and the channel without protein was used as a reference channel during interaction measurement. The reference channels enable monitoring of possible non-specific binding on the substrate.

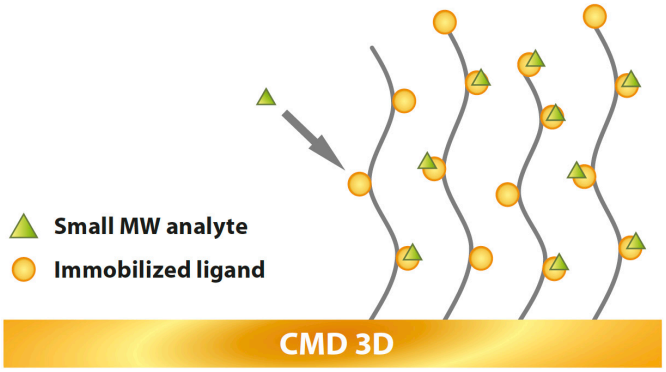

Figure 1. Small molecular weight drug interaction with protein.

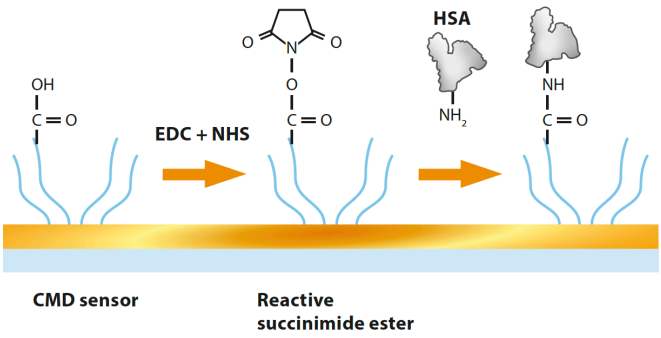

Figure 2. Ligand (human serum albumin, HSA) is amine coupled on a 3D dextran sensor surface (EDC/NHS activation of carboxyl groups) where analyte (indomethacin) interaction to the ligand is further measured.

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The interaction of a small molecular weight drug indomethacin (357.8 g/mol) with HSA was measured with the MP-SPR Navi™ 220A NAALI instrument. Seven indomethacin samples (0.1-50 µM) were measured at 21°C. Samples in pH 7.4 PBS (phosphate buffered saline) contained 3% of DMSO to enhance drug solubility. The kinetics and affinity of the interaction were analysed using TraceDrawer™ for MP-SPR Navi™.

Results and discussion

HSA immobilization was successfully performed *in situ* with amine coupling chemistry and using the BioNavis protocol. The amount of bound HSA was determined to be 1700 ng/cm^2 from the MP-SPR response.

Indomethacin binding constants (affinity and kinetic) towards HSA were calculated with appropriate binding models. Affinity and kinetic evaluations were performed with one-to-one binding models of TraceDrawer™ software (Figures 3 and 4). Dissociation constant (K_D) was in good agreement in both evaluations 1.41E-5 (concentration analysis) and 1.39E-5 M (kinetic evaluation). Association rate constant (k_a) was 2.45E3 (1/(M*s)) and dissociation rate constant (k_d) was 3.40E-2 (1/s).

Conclusions

MP-SPR is a reliable optical technique for molecule – molecule interaction analyses providing precise affinity and kinetic values of the interaction. Sensitive MP-SPR can be used for interaction analysis of small molecules. However, it is an excellent tool also for development of controlled drug release systems (nanoparticles) and optimisation of controlled drug release formulations (for further information see separate Application Notes #140 and #141).

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 420A ILVES or 220A NAALI

Sensor surface: SPR102-CMD-3D

Software: MP-SPR Navi™ Controller, DataViewer, and TraceDrawer™ for MP-SPR Navi™

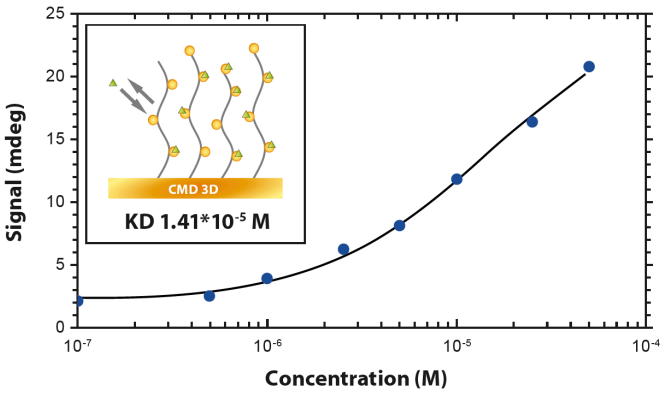

Figure 3. In the figure change KD to KD

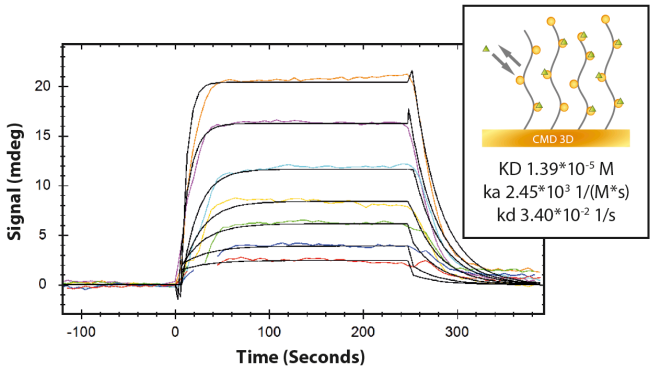

Figure 4. In the figure change KD, ka and kd to K_D, k_a and k_d

Reference:

Valtari *et al.*, Molecular Pharmaceutics Vol. 22 Issue 2, 2025.

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